Setting-up of a 24 h model to evaluate the activity of antibiotics against intracellular forms of *S. aureus* infection

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Why intracellular S. aureus ?

- facultative intracellular organism
  - macrophages, monocytes, PMN
  - endothelial cells …
- remains hidden for prolonged periods
- is supposedly protected against many antibiotics
  - exposed to concentrations that do not allow fast killing…
  - is in a metabolic situation that make less susceptible to antibiotics
- may be a cause of reccurence / resistance !!

Setting-up of the model : J774 mouse macrophages

- infection of macrophages (0.5 bact/cell)
- washing with gentamicin 50 µg/ml
- incubation for up to 24 h with
  - gentamicin 0.5 µg/ml (MIC) for controls
  - antibiotic under study alone for tests

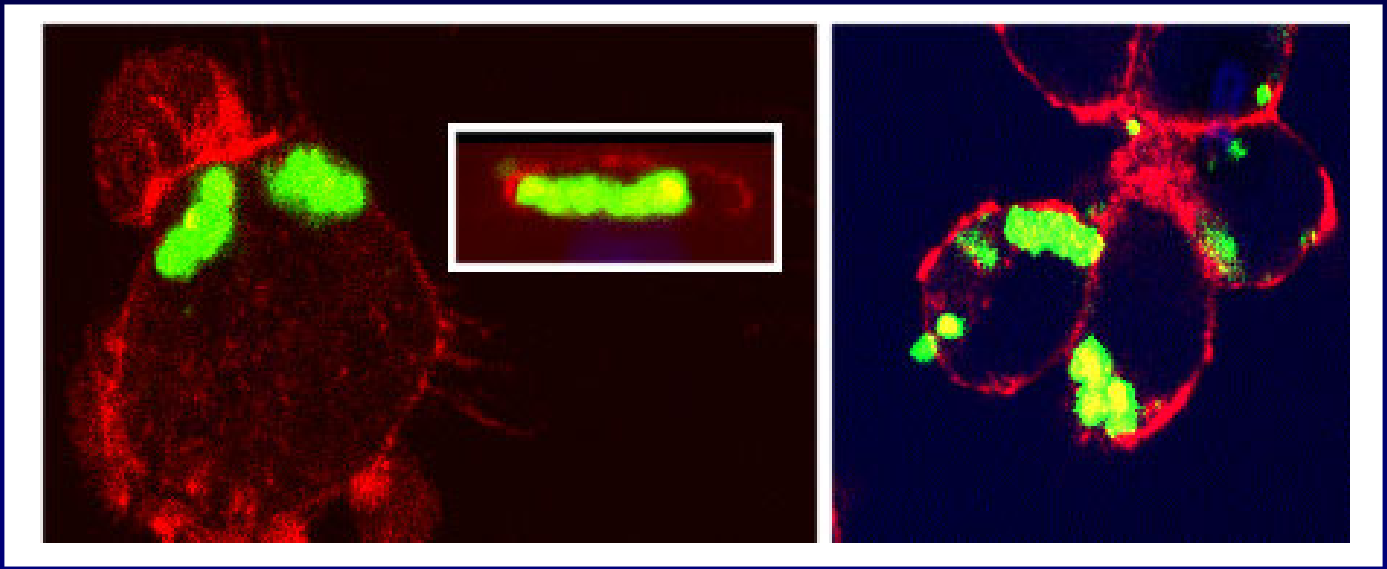
  ➔ the AB must be able to cope with both extracell. and intracell. *S. aureus* !!)

  at clinically-meaningfull concentrations (MIC to C_{max})

data from Seral *et al.* AAC 2003 47:2283-2292

Validation of the model : confocal microscopy

5 hours infection

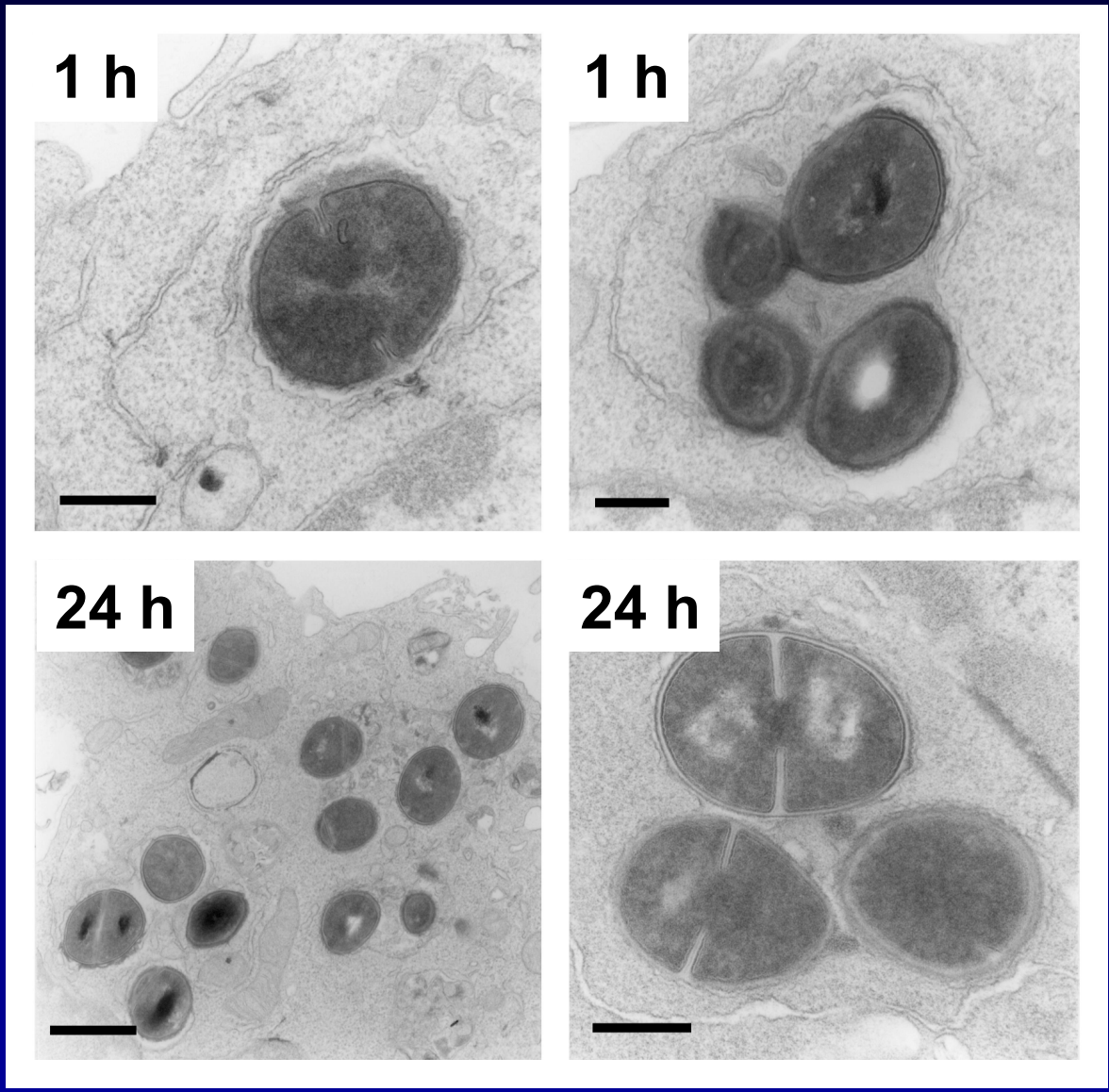


no antibiotic

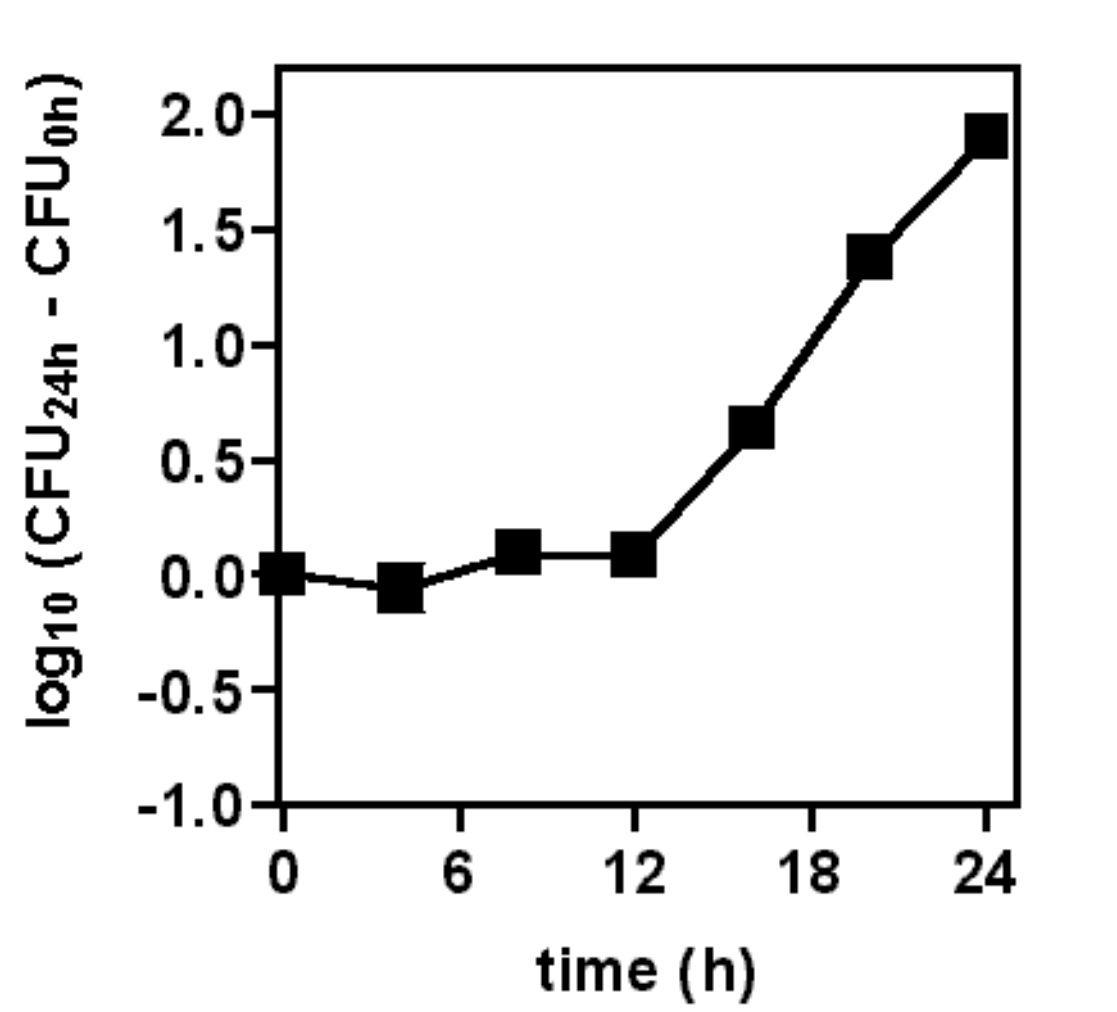
gentamicin 0.5 mg/L

data from Seral *et al.* AAC 2003 47:2283-2292

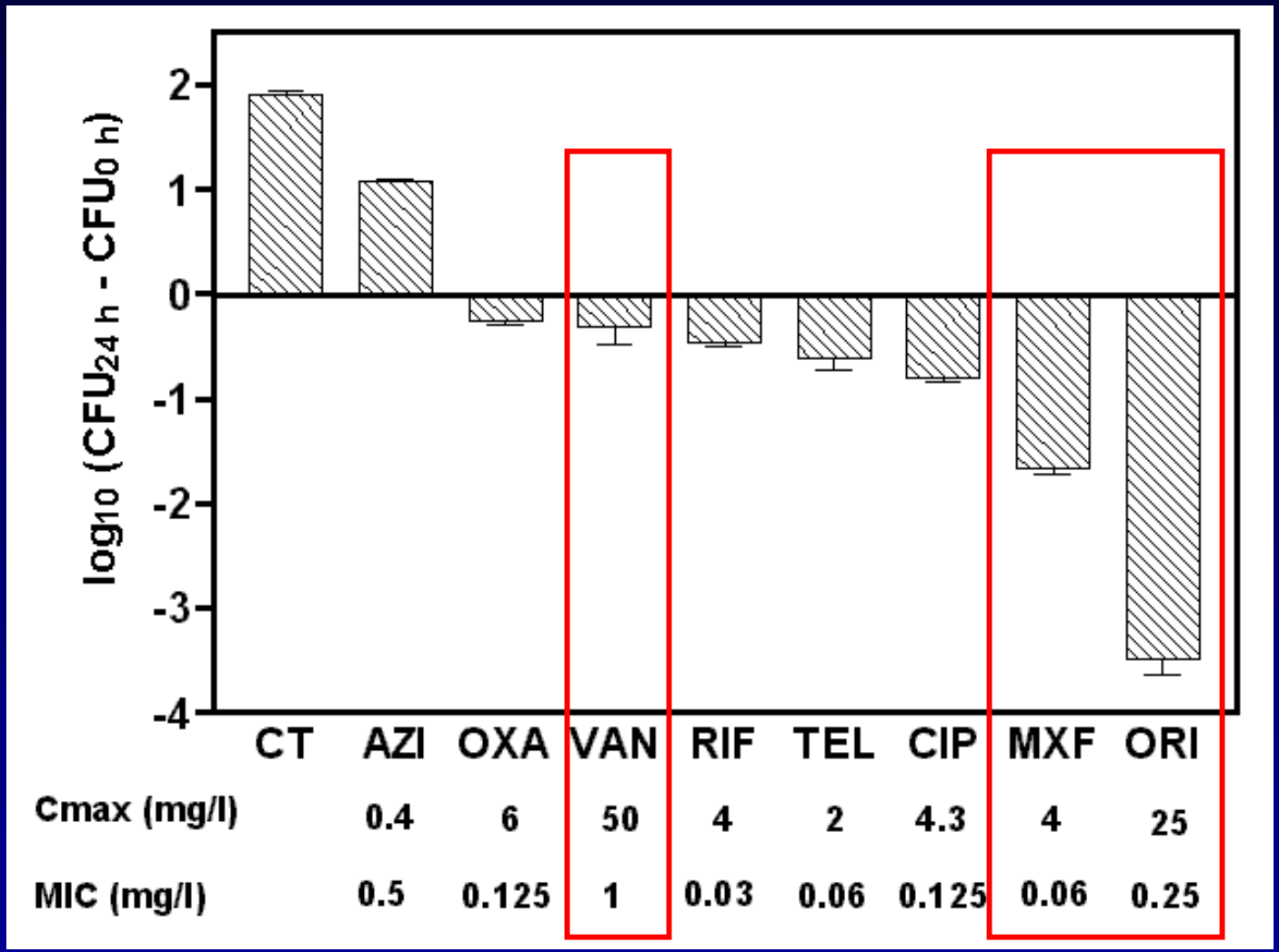
Validation of the model : electron microscopy



Validation of the model : S. aureus growth in controls



Use of the model to test antibiotic intracellular activity



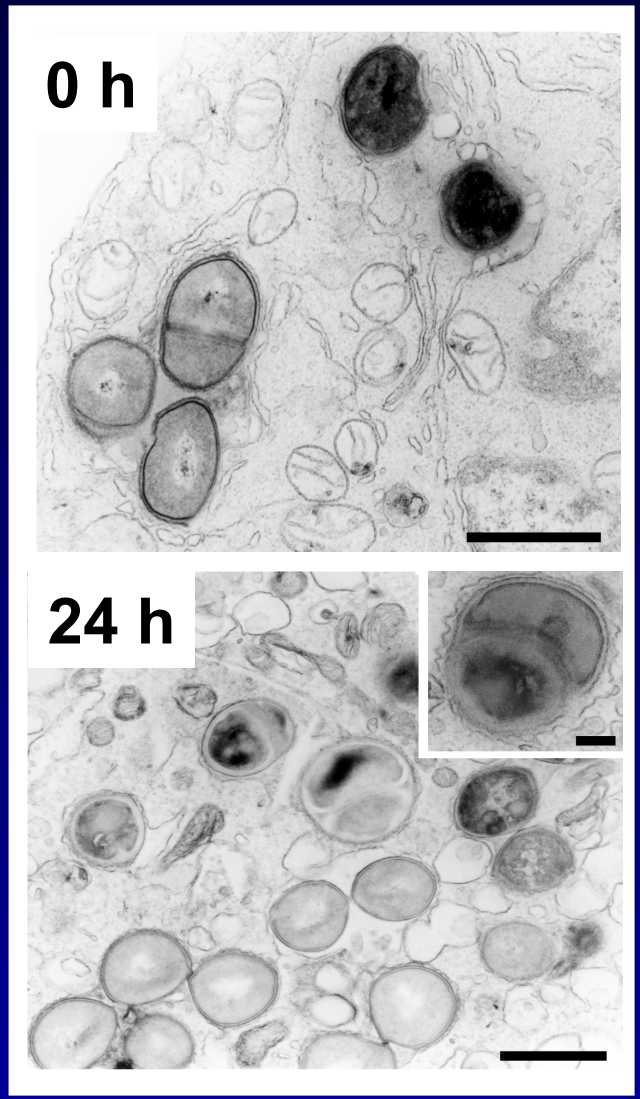
adapted from Seral *et al.* AAC 2003 47:2283-2292

An improved model : THP-1 human macrophages

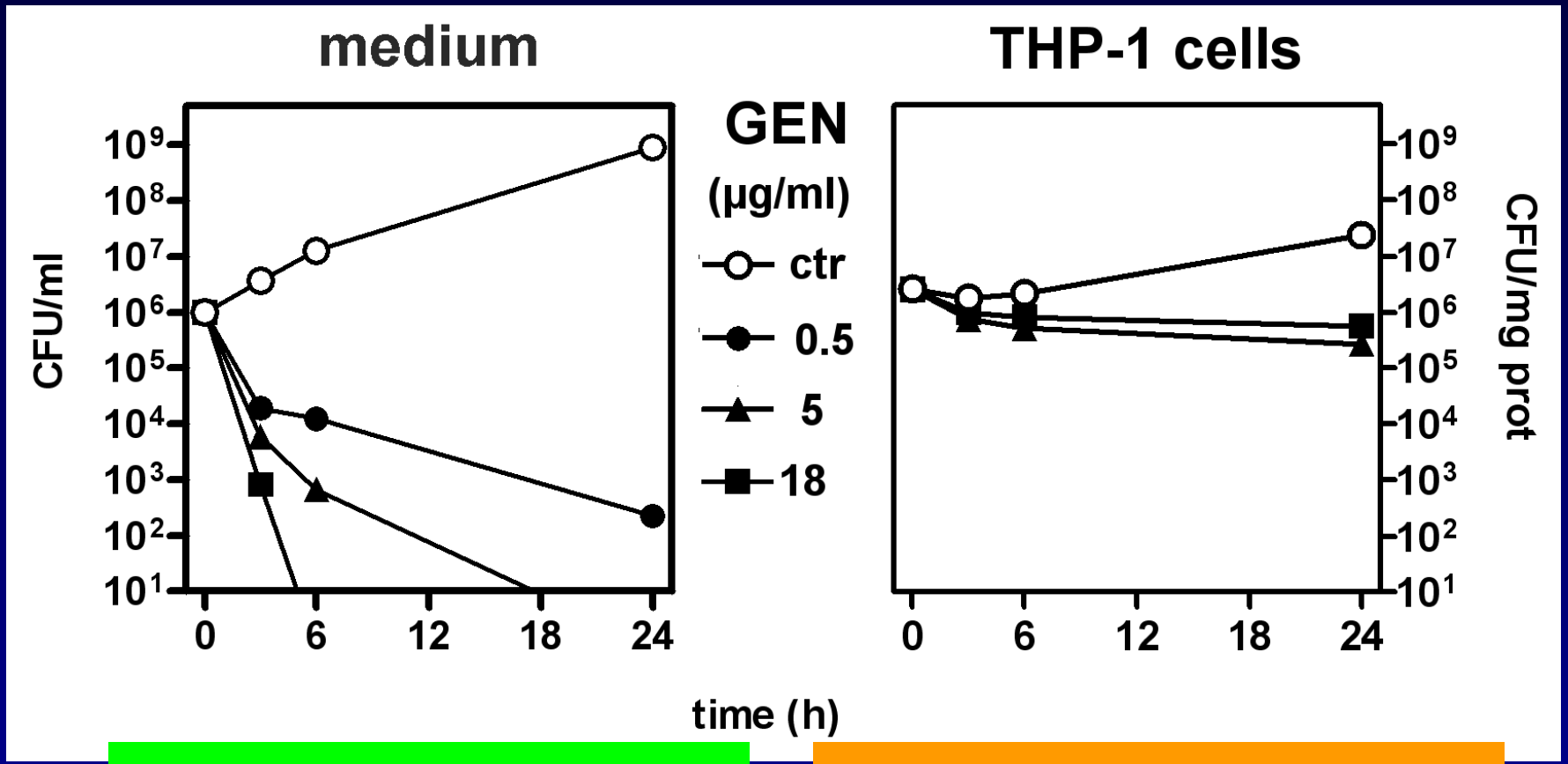
- behavior ~ as human monocytes
- valuable model for
  - AB activity testing
  - cytokine effect testing

Auwerx, Experientia, 1990 47:22-31

- infection of macrophages (4 bact/cell)
- washing with GEN 50 µg/ml
- incubation for up to 24 h with
  - GEN 0.5 µg/ml (MIC)
  - antibiotic under study



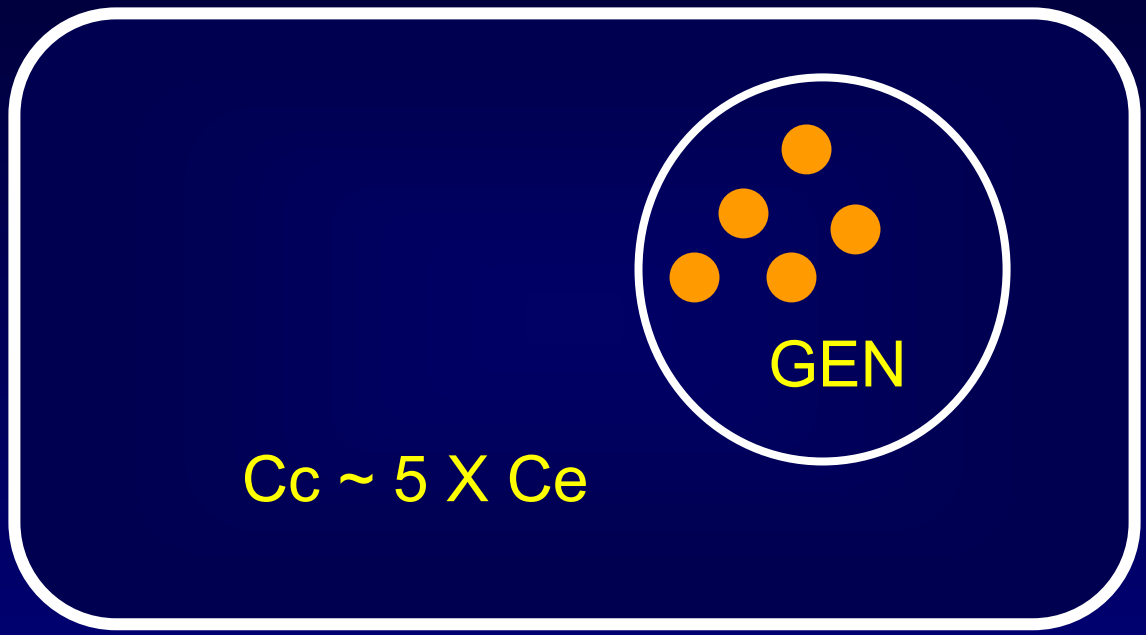
Aminoglycoside activity : concentration-effect relation for gentamicin



- highly bactericidal
- concentr. dependent

- Poorly bactericidal
- concentr. independent

Aminoglycoside activity : possible reasons for loss of activity



PHARMACOKINETIC PARAMETERS

2 Accumulation

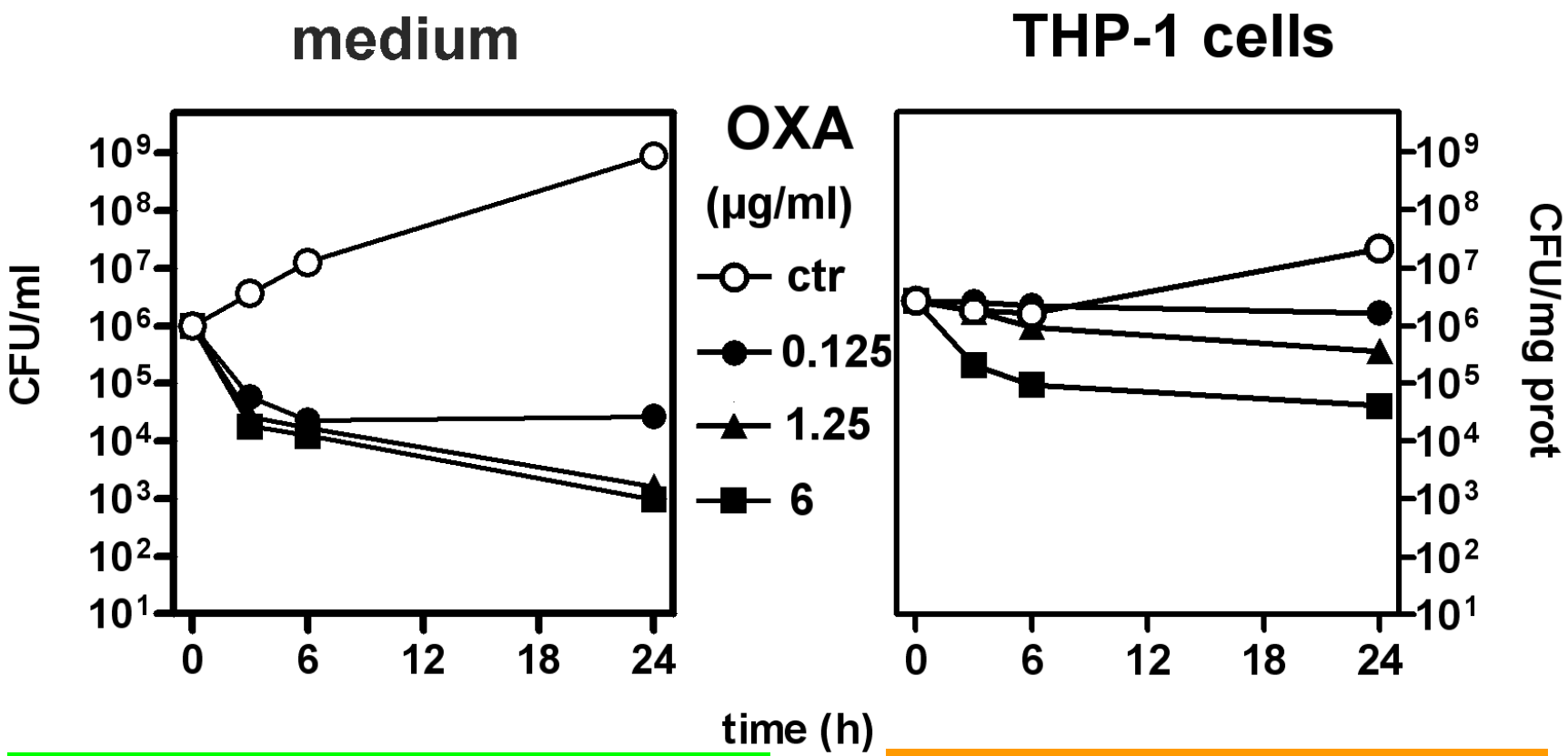
too slow ? Probably NO !
(preloading ineffective …)

PHARMACODYNAMIC PARAMETERS

5 Expression of activity

acidity ? YES !
MIC pH 5 = 32 X MIC pH 7

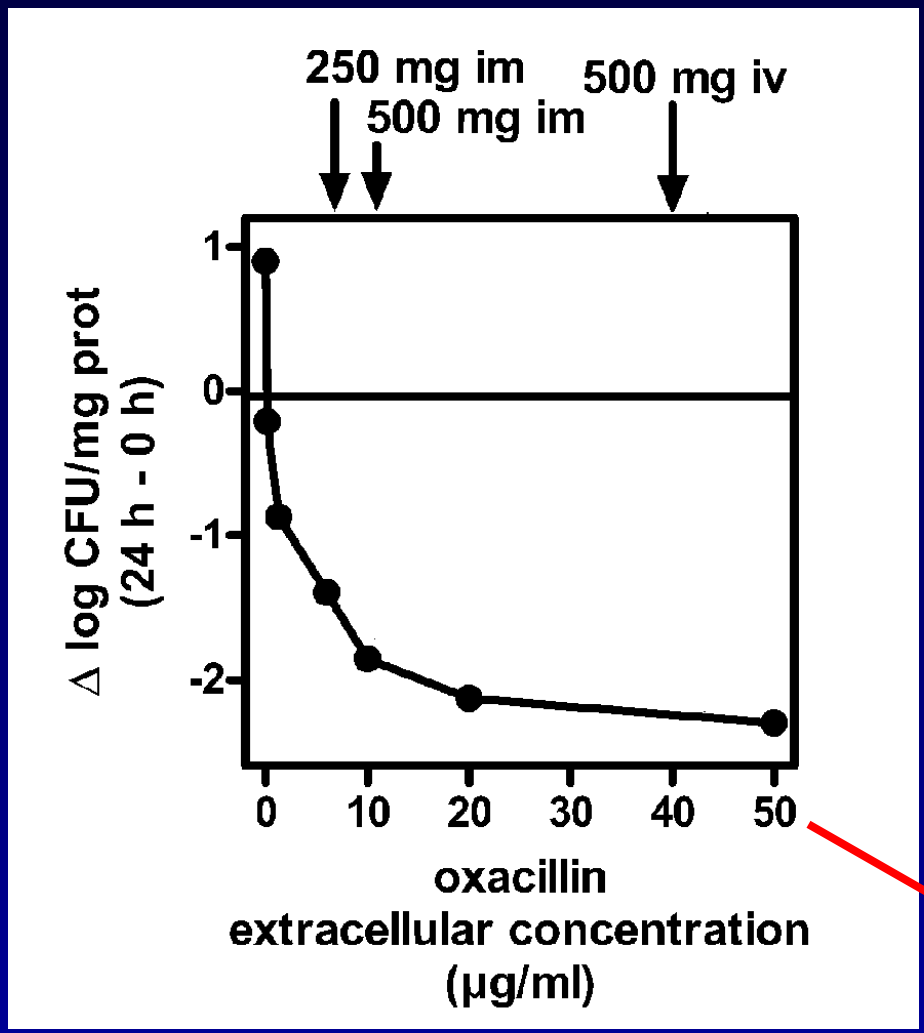
Beta-lactam activity : concentration-effect relation for oxacillin



- More slowly bactericidal
- concentr. independent

- slowly acting
- concentr. dependent

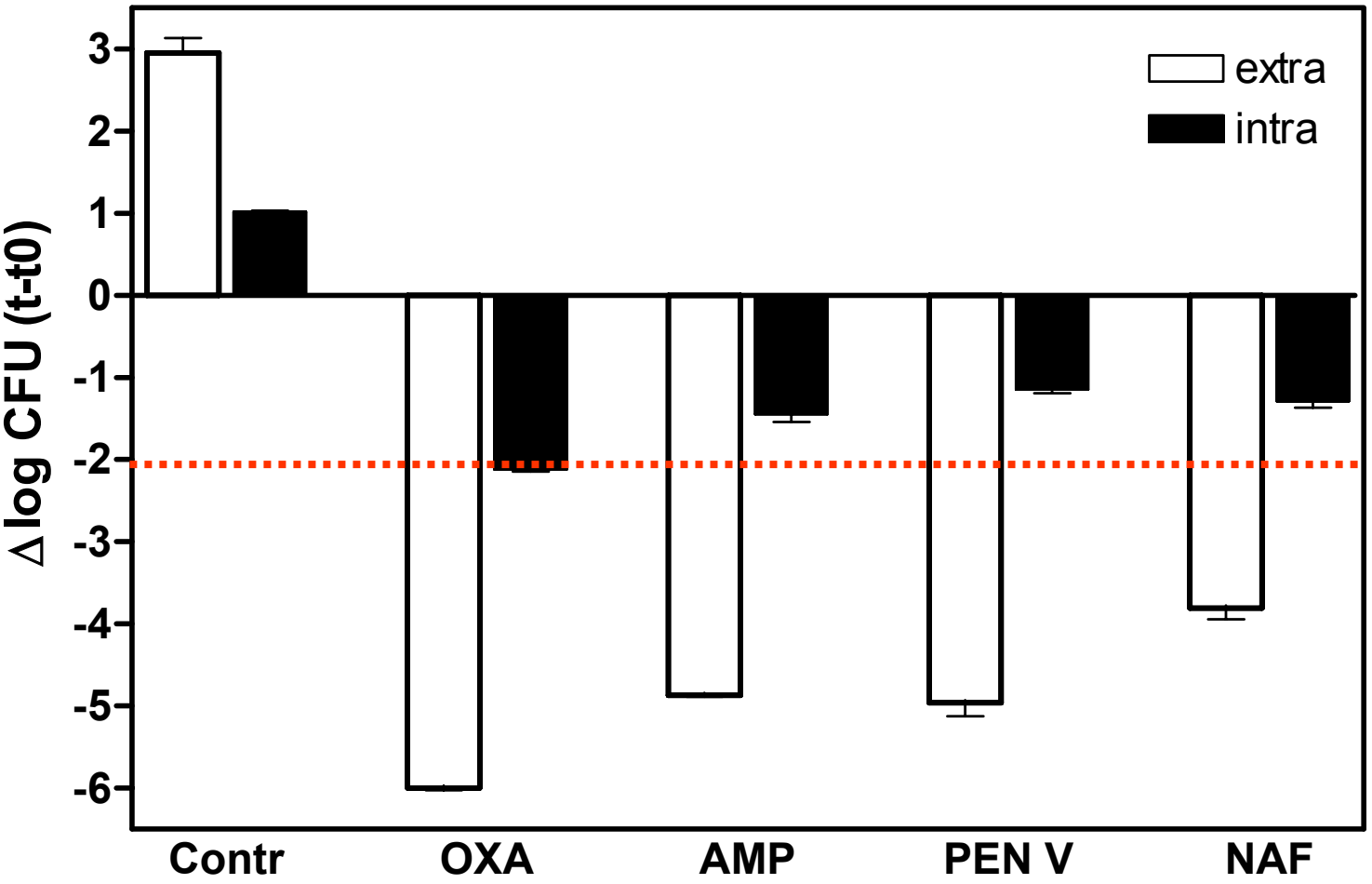
Beta-lactam activity : concentration-effect relation for oxacillin and relation to clinical dosings



Intracellular (?) β-lactams are concentration-dependent down to 2 log killing !!

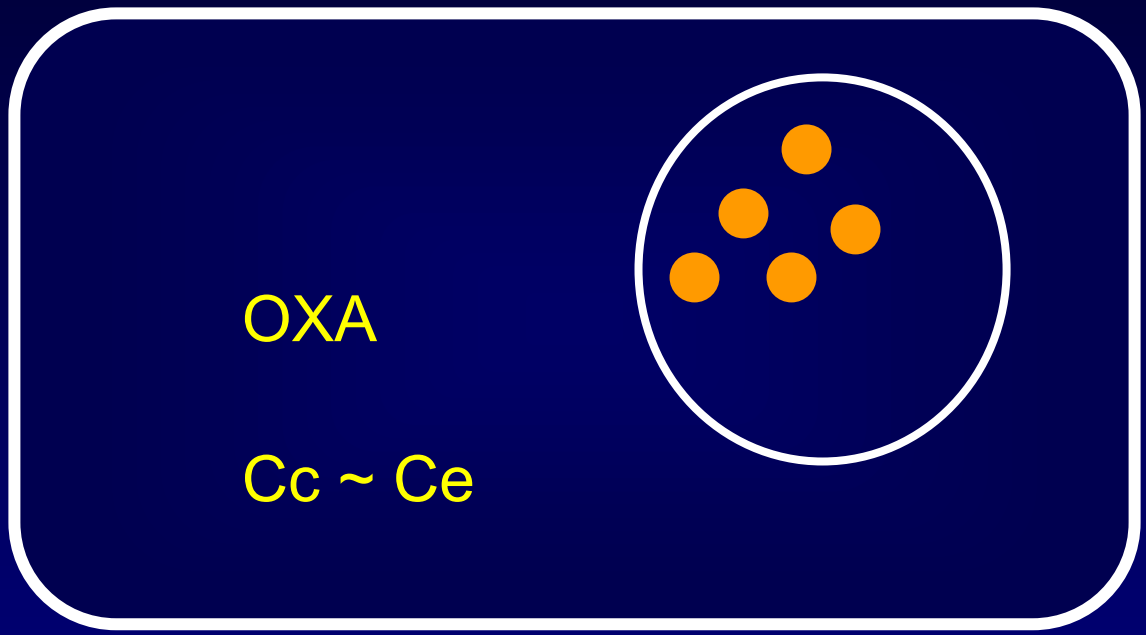
400 X MIC !

Beta-lactam activity at Cmax



MIC (ug/ml)	0.125	0.06	0.015	0.25
Cmax (ug/ml)	63	48	6.3	40
Accumulation	< 1	<1	< 1	<1

Oxacillin activity : possible reasons for (loss of) activity



PHARMACOKINETIC PARAMETERS

2 Accumulation
could be compensated by higher Ce …

3 Subcellular distribution
partial redistribution ?

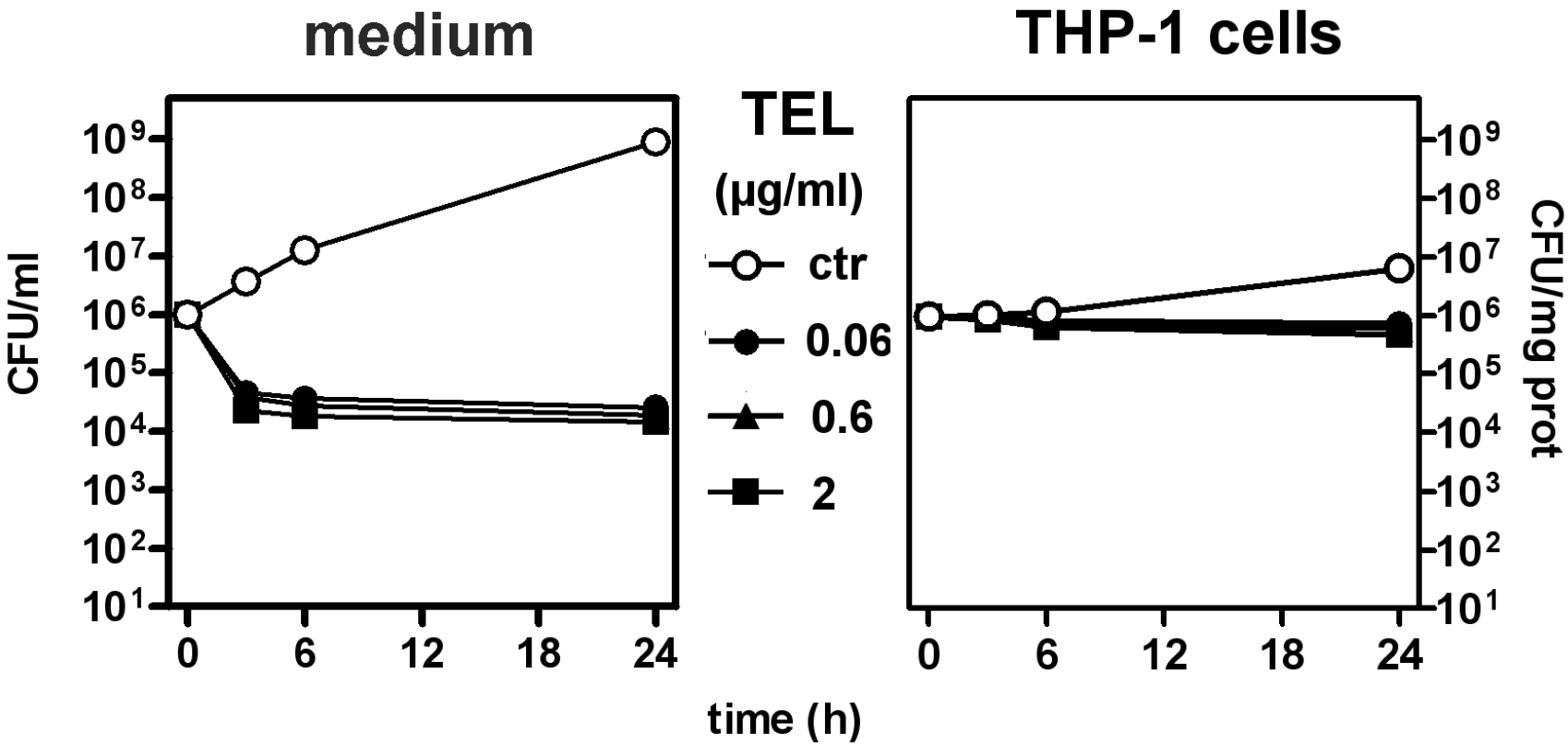
PHARMACODYNAMIC PARAMETERS

6 Bacterial responsiveness
slowly growing bacteria …

7 Cooperation with host defenses
was proposed to explain activity*

* Van den Broek *et al.* JAC 1986 17: 767-774

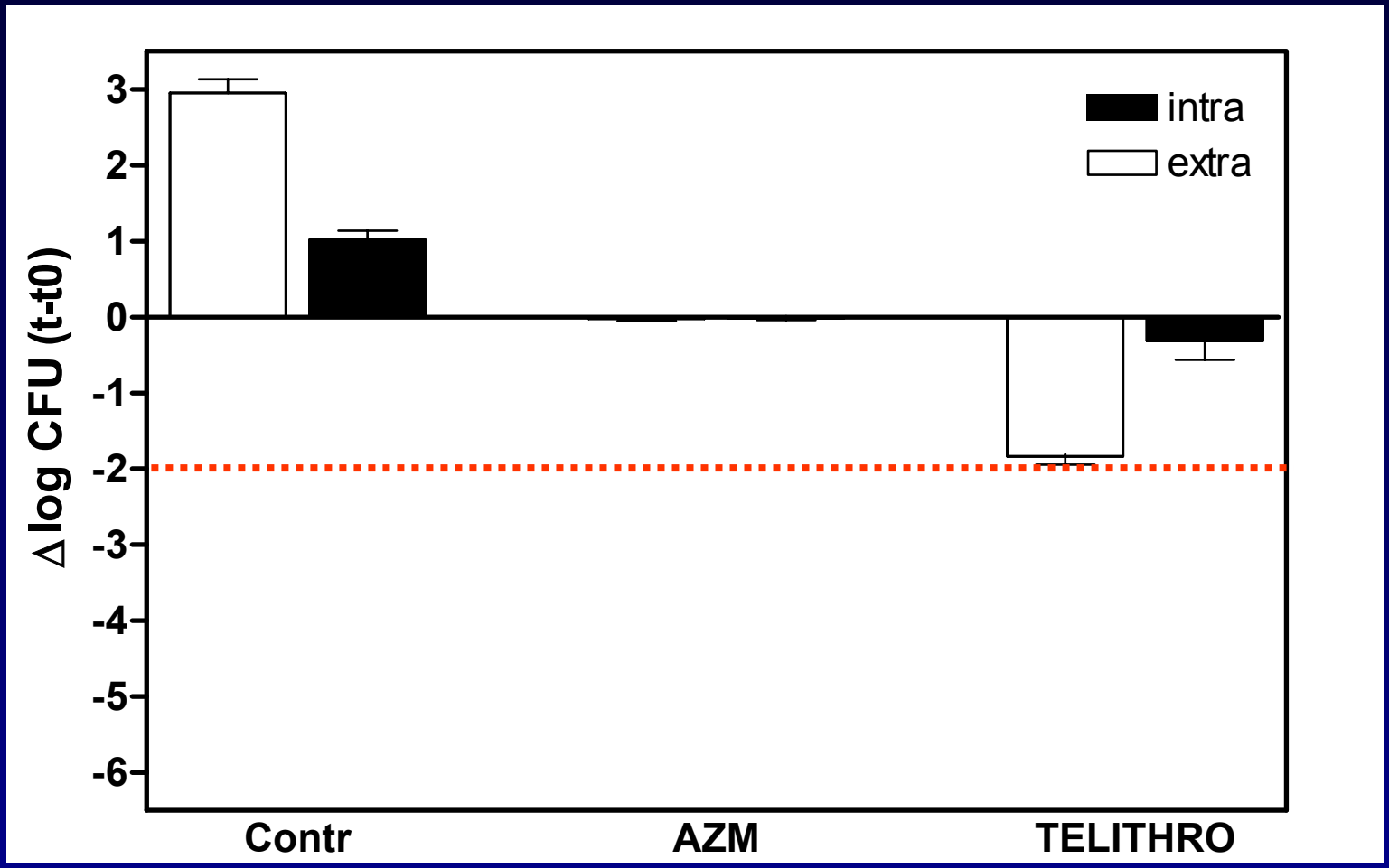
Macrolide activity : concentration-effect relation for telithromycin



- Limit. bactericidal effect
- concentr. independent

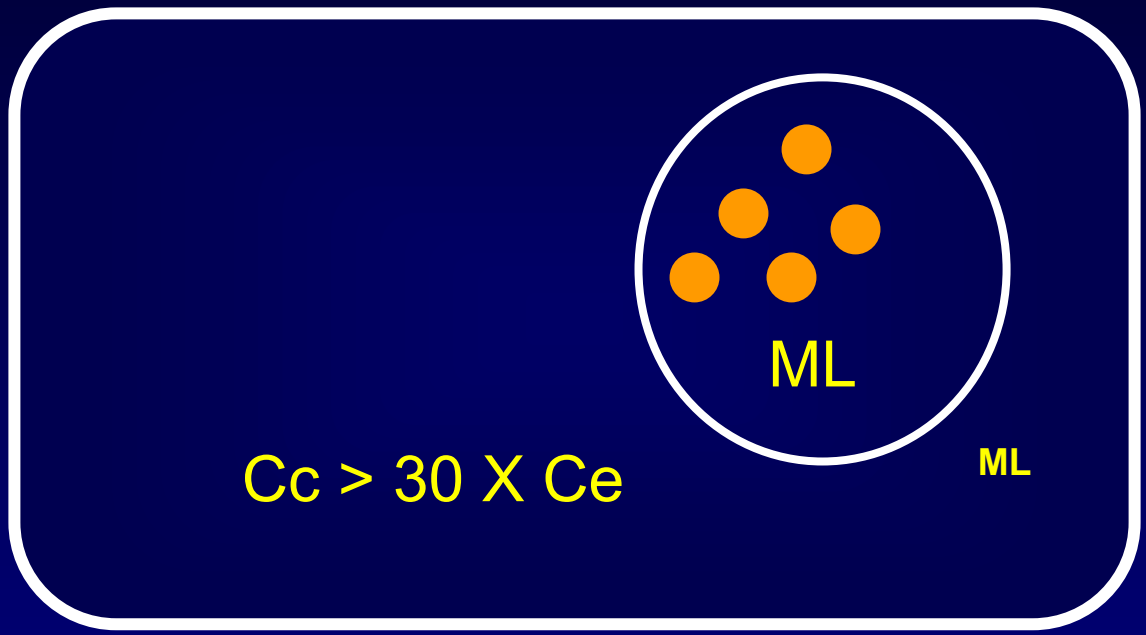
- static only
- concentr. independent

Macrolide activity at Cmax



MIC (ug/ml)	0.5	0.06
Cmax (ug/ml)	0.5	2
Accumulation	38	28

Macrolide activity : possible reasons for loss of activity



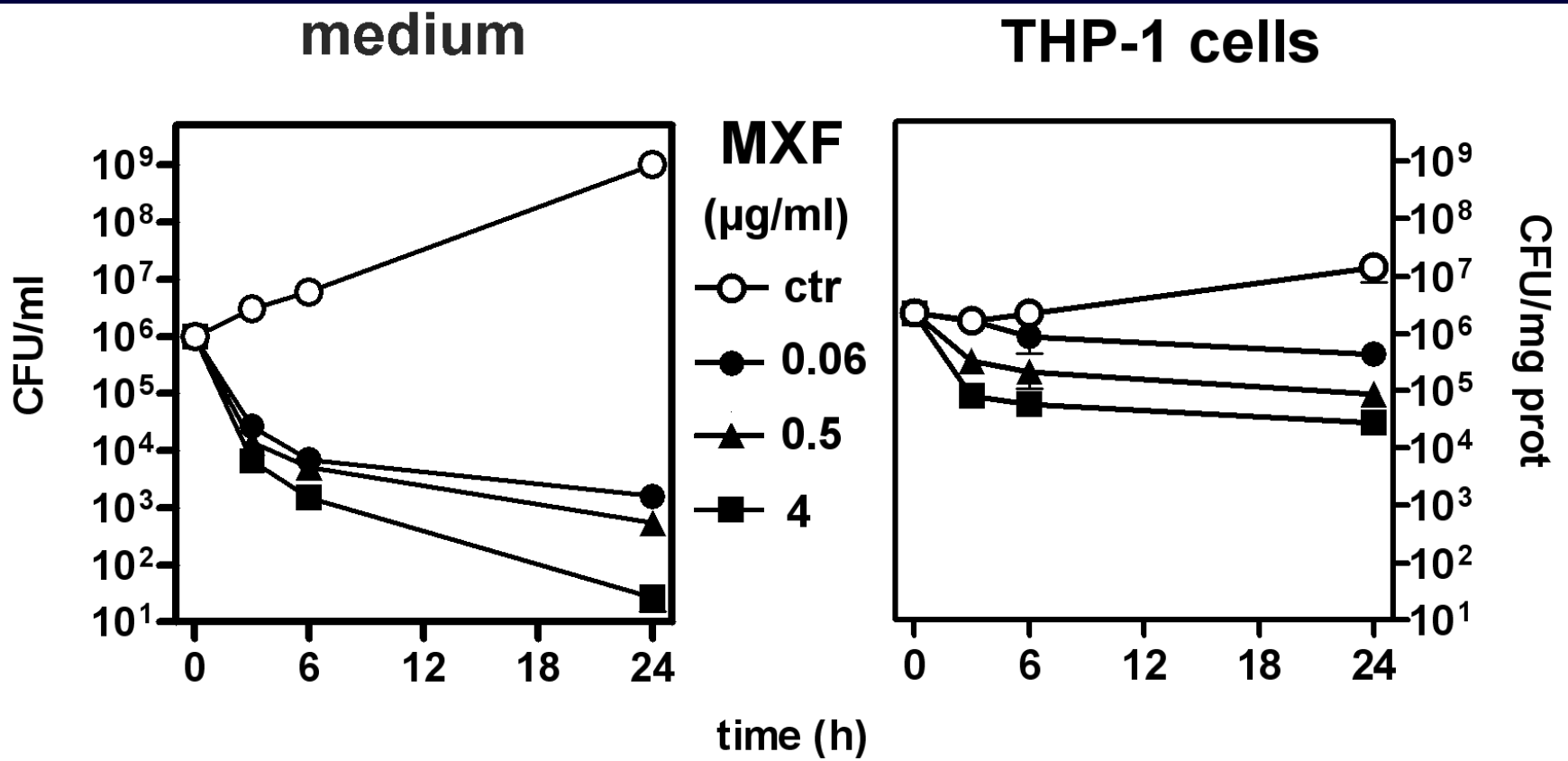
PHARMACOKINETIC PARAMETERS

PHARMACODYNAMIC PARAMETERS

5 Expression of activity

acidity ? YES !
MIC pH 5 = 100 X MIC pH 7
bacteriostatic

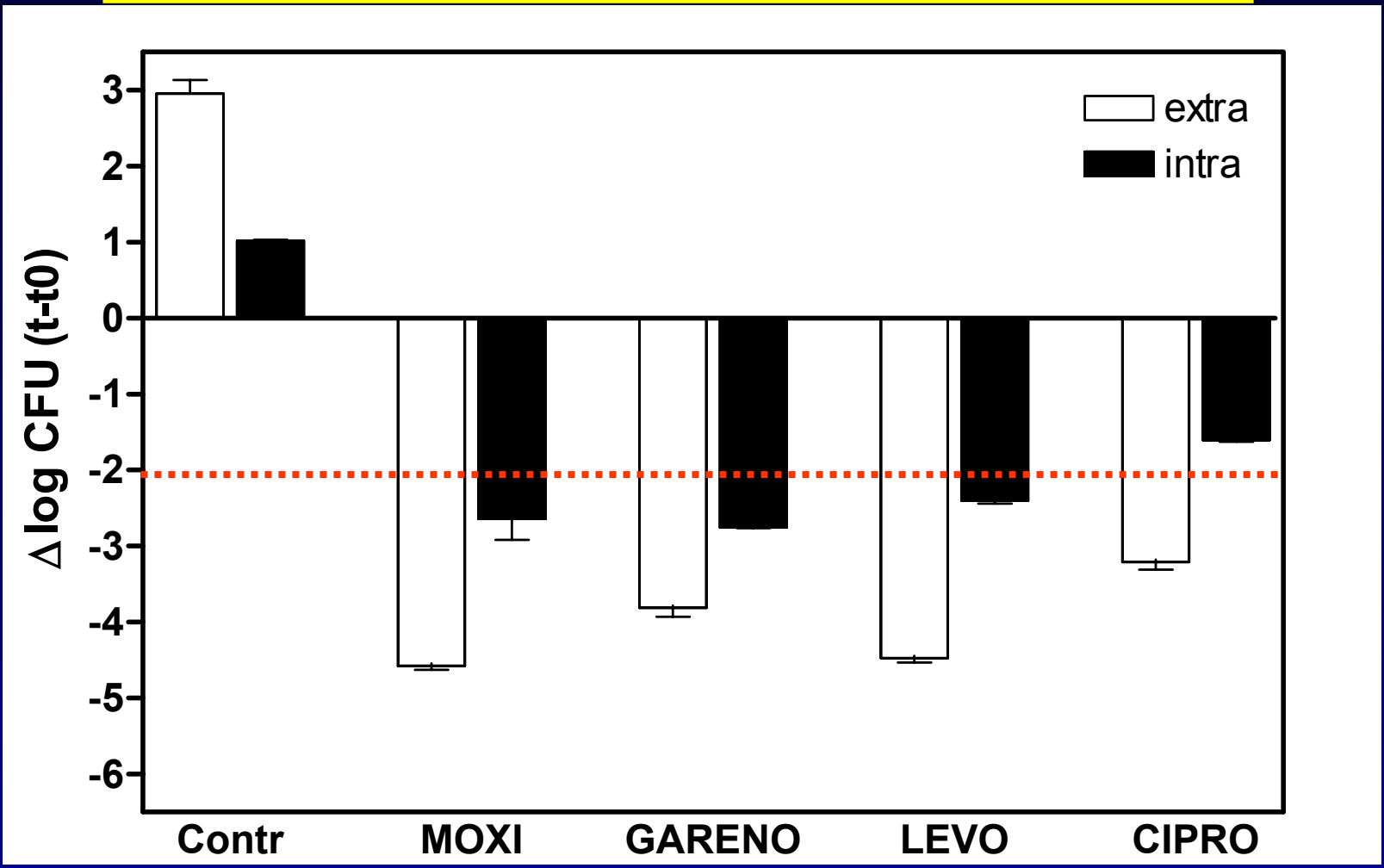
Quinolone activity : concentration-effect relation for moxifloxacin



- bactericidal
- concentr. dependent

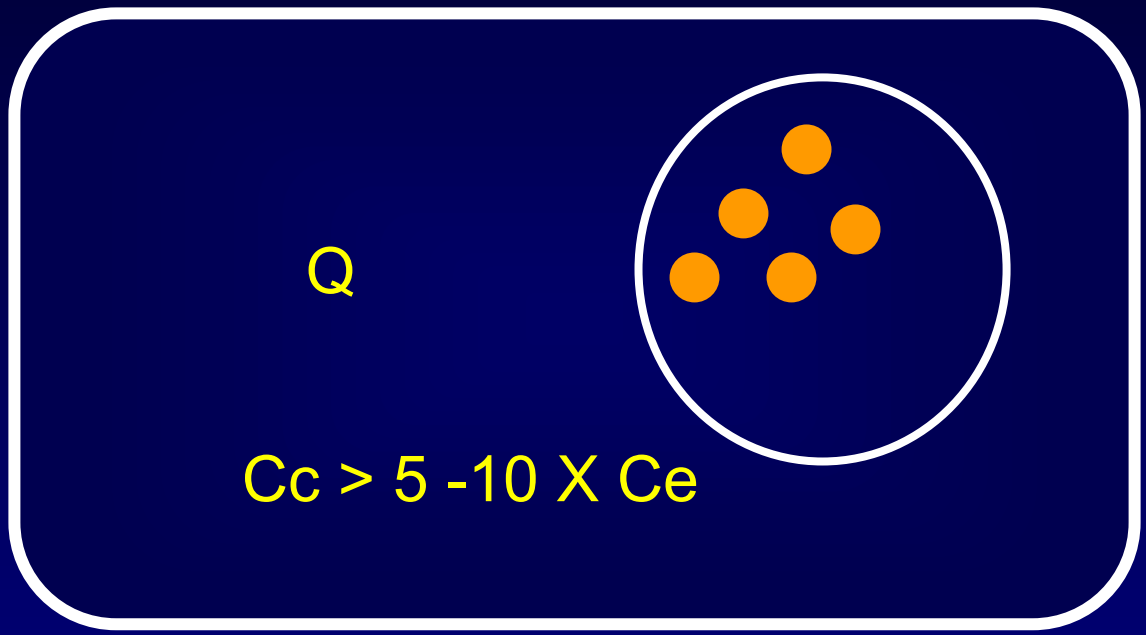
- concentr. dependent
- but more slowly acting
- and limited to 2-3 log

Quinolone activity at Cmax



MIC (ug/ml)	0.06	<0.03	0.125	0.125
Cmax (ug/ml)	4	4	4	4.3
Accumulation	8	9	7	5

Quinolone activity : possible reasons for reduced activity



PHARMACOKINETIC PARAMETERS

3 Subcellular distribution

Soluble fraction ?

Highly diffusible and relocalisable

4 Inactivation

Binding to cell constituents ?

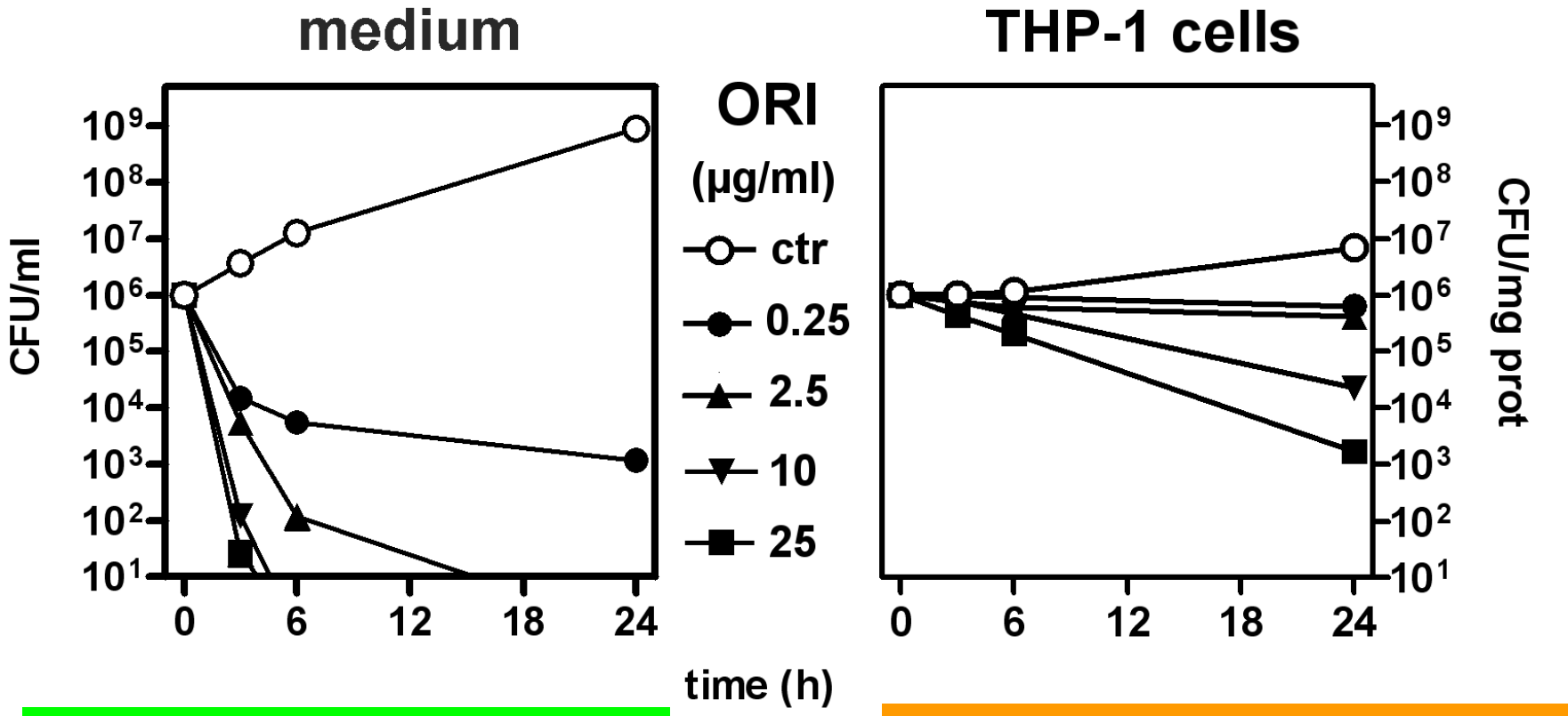
PHARMACODYNAMIC PARAMETERS

5 Expression of activity

acidity ? YES !

MIC pH 5 = 4-8 X MIC pH 7

Oritavancin : concentration-effect relationships

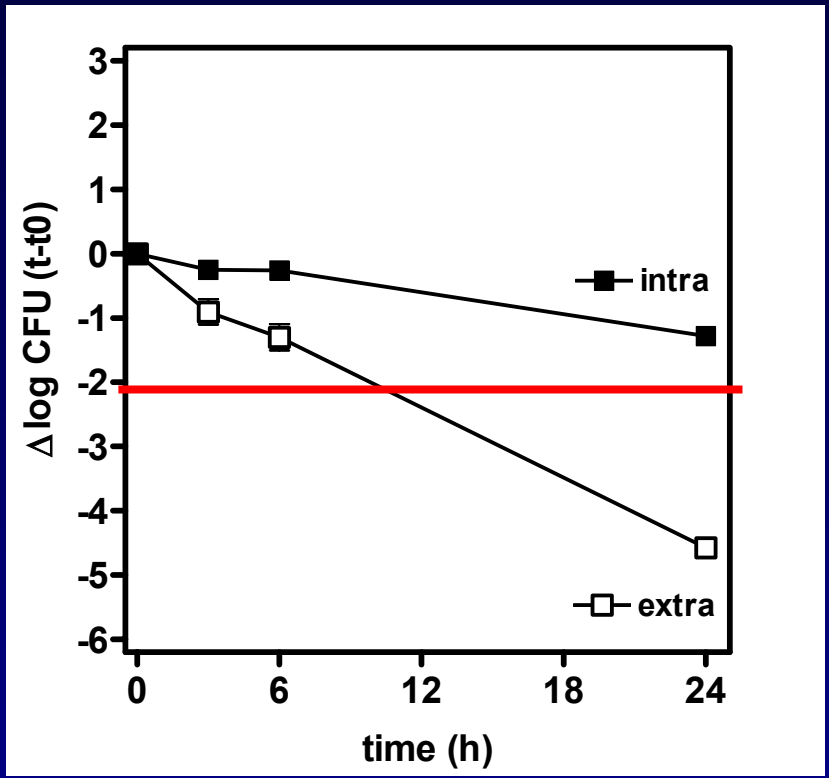


- highly bactericidal
- concentr. dependent

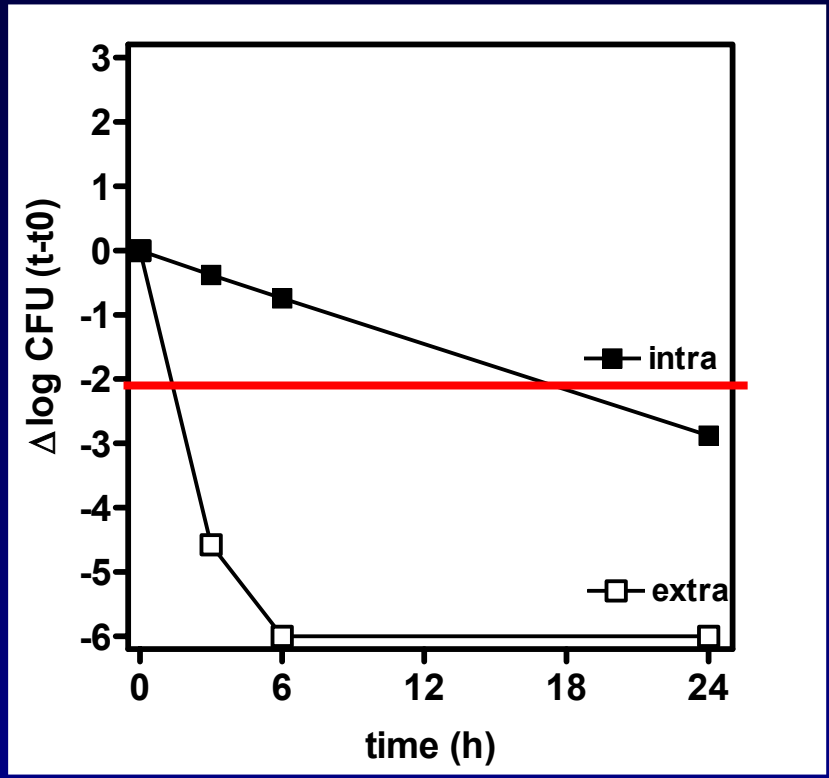
- more slowly acting
- but still bactericidal
- and concentr. dependent

Comparison vancomycin - oritavancin

vancomycin



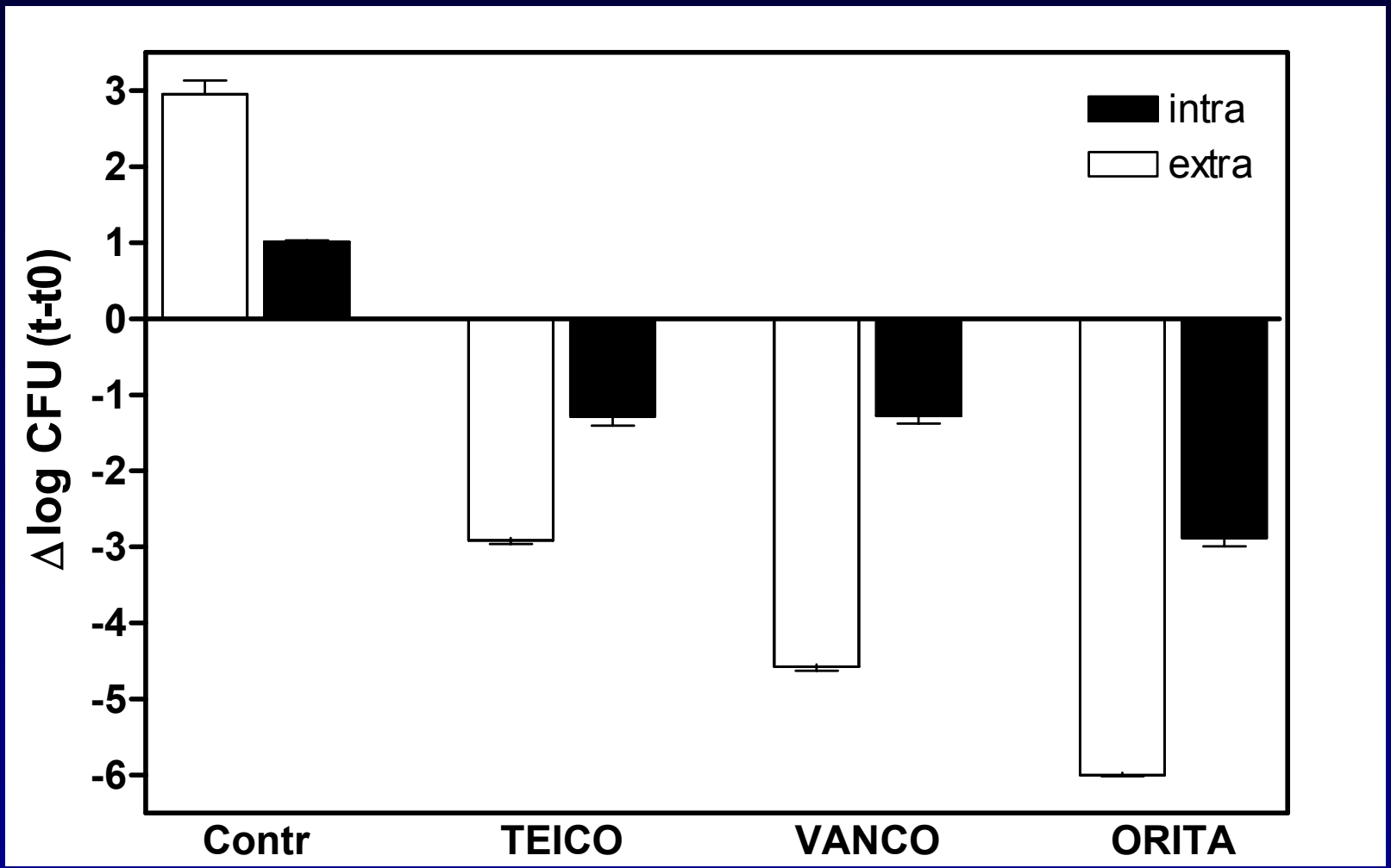
oritavancin



Slowly cidal extracellularly mostly static intracellularly

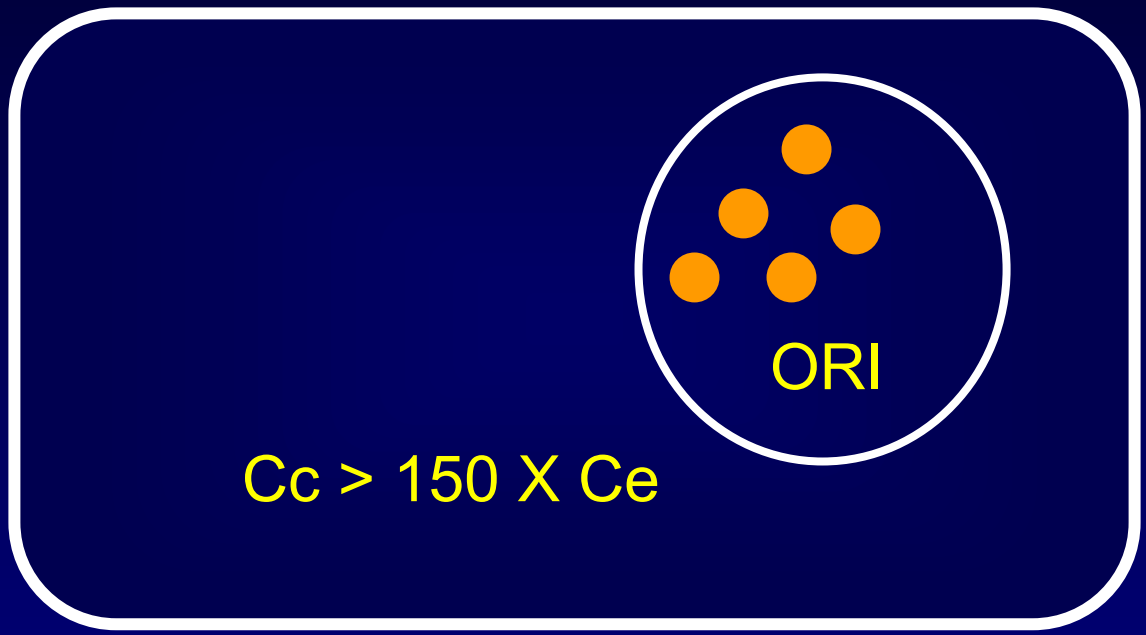
Rapidly cidal extracellularly; More slowly but still cidal intracellularly at 24h

Glycopeptide activity at Cmax



MIC (ug/ml)	0.25	1	0.25
Cmax (ug/ml)	100	50	25
Accumulation	7	6	148

Oritavancin activity : possible reasons for loss of activity



PHARMACOKINETIC PARAMETERS

4 Inactivation

Binding to cell constituents ?

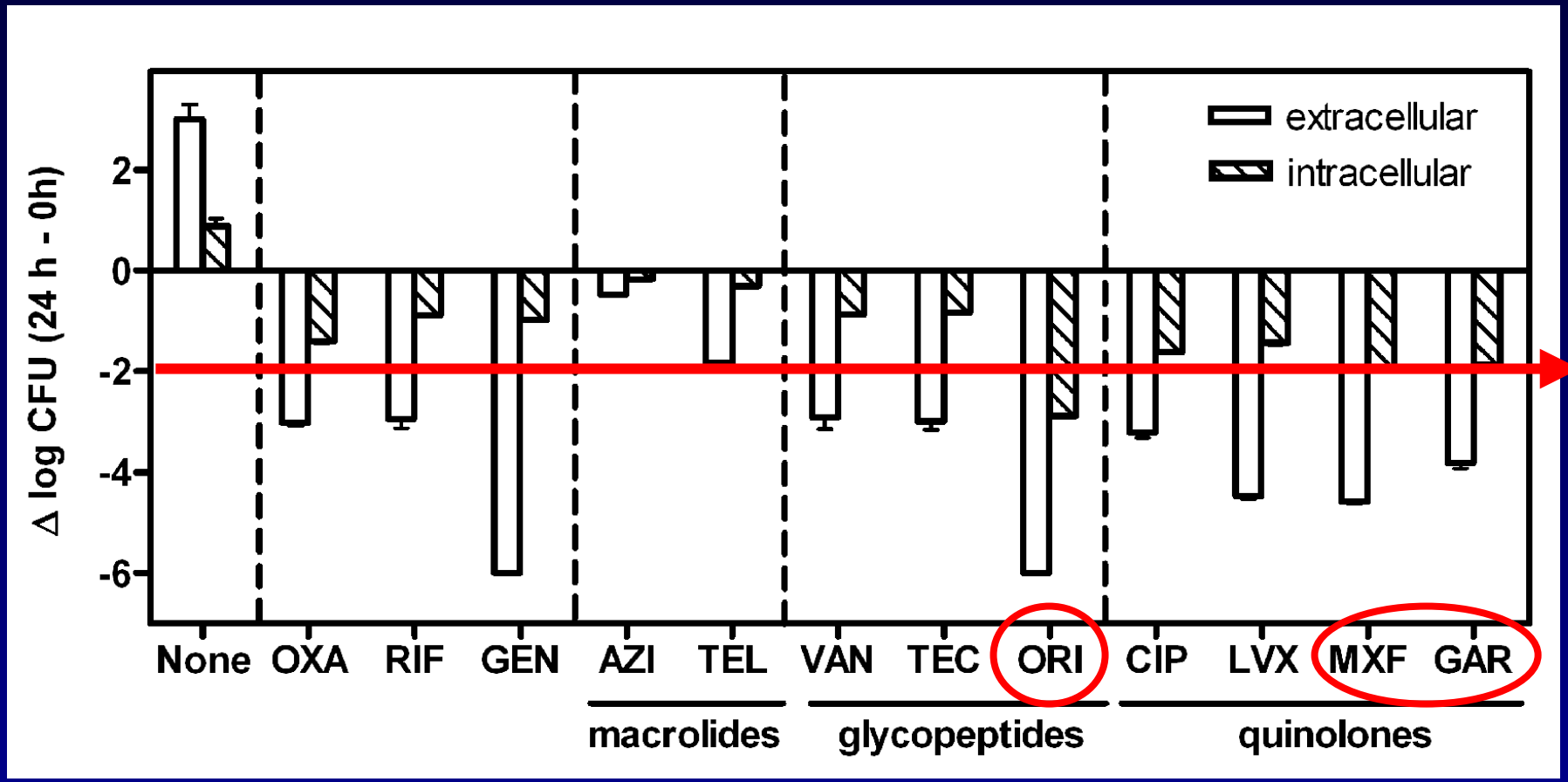
PHARMACODYNAMIC PARAMETERS

5 Expression of activity

acidity ? NO !

MIC pH 5 = MIC pH 7

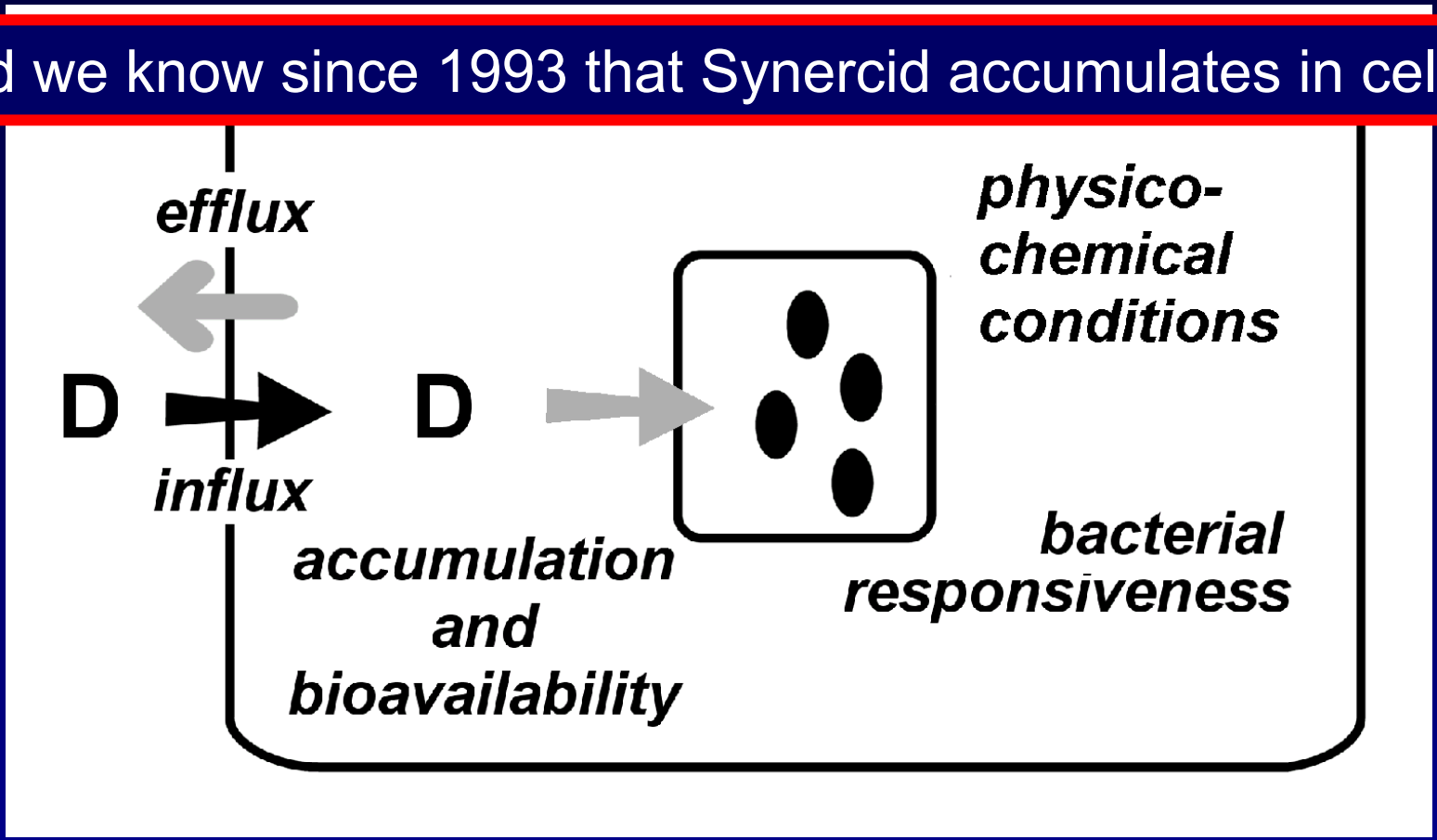
Antibiotic activity : summary



NCCLS definition of bactericidal activity

PK – PD parameters predictive of intracellular activity: a global view

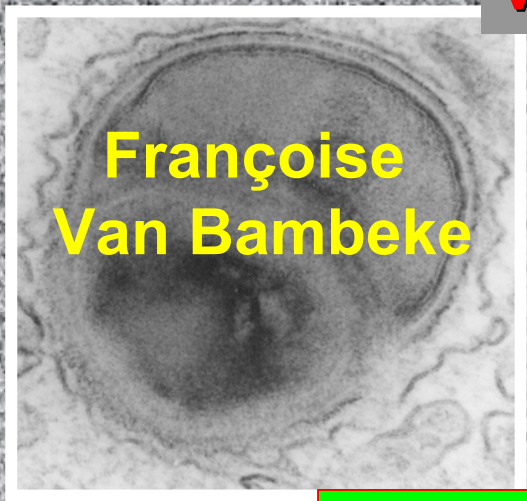
And we know since 1993 that Synercid accumulates in cells ...

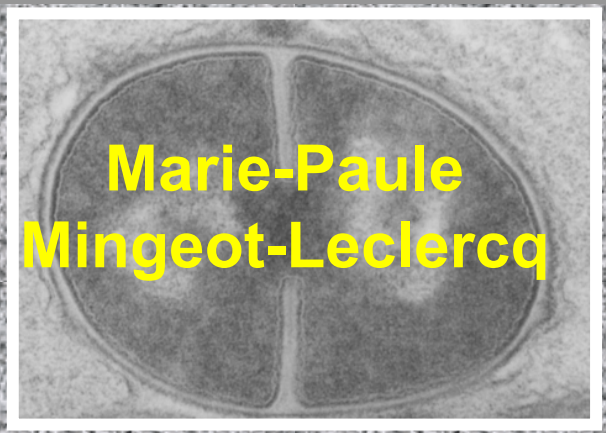


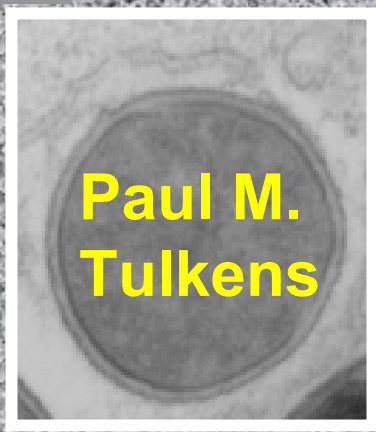
adapted from Carryn *et al* 2003, Infect Dis Clin N Am

And here is the team….

visit www.antiinfectieux.org







And visit also the ISAP web site: www.isap.org